ISSN (print) 0093-4666 © 2011. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

Volume 116, pp. 203–216 *April–June 2011*

DOI: 10.5248/116.203

Spiroplana centripeta gen. & sp. nov., a leaf parasite of *Philadelphus* and *Deutzia* with a remarkable aeroaquatic conidium morphology

Hermann Voglmayr[1*], Mi-Jeong Park[2] & Hyeon-Dong Shin[2*]

[1]*Department of Systematic and Evolutionary Botany, University of Vienna, Rennweg 14, A-1030 Vienna, Austria*

[2]*Division of Environmental Science and Ecological Engineering, College of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Korea*

*Correspondence to: *hermann.voglmayr@univie.ac.at* & *hdshin@korea.ac.kr*

Abstract — *Spiroplana centripeta* is described as a new genus and species from Korea. Its spirally coiled conidia closely resemble those of the aeroaquatic genus *Spirosphaera* but its ecology differs totally, as it is parasitic to living *Philadelphus* and *Deutzia* leaves causing symptoms superficially similar to powdery mildew disease. No sexual state has been found, but molecular phylogenies inferred from ITS1-5.8S-ITS2 rDNA and partial nuLSU sequences support it within the *Pleosporales* (*Dothideomycetes*) and thus phylogenetically distinct from the generic type, *Spirosphaera floriformis* in the *Leotiomycetes*. Molecular phylogenies further show that *Spirosphaera* is polyphyletic and that generic diagnostic characters have evolved multiple times as an adaptation to conidium dispersal in the aeroaquatic niche. Morphologically, *Spiroplana centripeta* differs from *Spirosphaera* in its branching pattern, characterised by a main coil of cells in the conidial filament that give rise to 1–2 daughter filaments only on the inner side of the main coil. The daughter filaments then grow, coiling inwards with occasional additional branching to produce a tightly interwoven propagule enclosing air in a manner similar to aeroaquatic fungi. As primary branching takes place in one plane, the conidia are laterally flattened. In light of these molecular, morphological and ecological differences, a new genus is described. We believe the astounding similarity of the *Spiroplana* and *Spirosphaera* conidia is related to dispersal on the surface of a water film.

Key words — leaf pathogen, *Philadelphaceae*, taxonomy

Introduction

During an inventory on plant parasitic fungi of South Korea, a remarkable fungal leaf parasite was found on living *Philadelphus* and *Deutzia* (*Philadelphaceae*) leaves. Although superficially similar to a powdery mildew by forming a whitish, cobweb-like mycelium on the lower leaf epidermis, microscopical investigations revealed complex multicellular conidia most

similar to those of the aeroaquatic anamorph genus *Spirosphaera* Beverw. However, the latter genus comprises solely saprobic aquatic species inhabiting organic litter submerged in stagnant to slow-flowing water bodies (Voglmayr 2004) and is therefore ecologically distinct; in addition, the present fungus did not fit any described *Spirosphaera* species.

Subsequently, detailed molecular phylogenetic and morphological investigations were initiated to reveal the systematic and phylogenetic affiliations of the *Spirosphaera*-like leaf pathogen. As a result, a new genus and species are described, acknowledging its morphological, phylogenetic and ecological distinctness from *Spirosphaera*.

Materials & methods

Sampling

During 2004–2010 in Korea, 23 samples of a *Spirosphaera*-like fungus were collected from *Philadelphus schrenkii* and three from *Deutzia parviflora*. The fungus occurred on host plants growing in shady humid sites located mostly near streams, and it was collected from July to October, especially in the monsoon season. Herbarium samples of dried infected leaves are deposited at KUS and WU. Type or authentic cultures of *Spirosphaera beverwijkiana* Hennebert (CBS 469.66), *S. floriformis* Beverw. (CBS 402.52), and *S. minuta* Hennebert (CBS 476.66) were selected from the CBS (Utrecht, The Netherlands) for sequencing.

Light microscopy

Descriptions and measurements of cellular structures were based mainly on dried specimens removed directly from the infected leaves and mounted in tap water or L4 (general mounting fluid after Clémençon 1972) for light microscopy. Slides were examined and photographed using a Zeiss Axio Imager.A1 (Zeiss, Jena, Germany) microscope equipped with a Zeiss AxioCam ICc3 digital camera. Measurements are reported as maxima and minima in parentheses and the mean plus and minus the standard deviation of a number of measurements given in parentheses.

Scanning electron microscopy

Fresh infected leaf samples were prepared for scanning electron microscopy (SEM) according to the method described in Halbritter (1998). The prepared specimens were mounted on Cambridge stubs, sputter-coated with gold, and examined in a Jeol JSM-6390LV scanning electron microscope (Jeol Ltd., Japan) at 10 kV and a Zeiss Supra 55VP field-emission scanning electron microscope (Carl Zeiss, Germany) at 2 kV.

Cultures

Conidia were scraped off the leaf lesion into a drop of sterile distilled water to prepare a conidial suspension, which was streaked onto potato dextrose agar (PDA) supplemented with streptomycin sulphate (200 ppm). After germination, each conidium was transferred on PDA and grown at 25°C under a 12-hr photoperiod. Five monoconidial isolates were successfully obtained in the present study. All the isolates, listed in Table 1, were deposited at the KACC (Korean Agricultural Culture Collection, Suwon, Korea).

Table 1. Information on the *Spiroplana centripeta* isolates cultured and sequenced.

Host	Isolate no.	Voucher specimen no.	ITS, LSU GenBank acc. no.
Philadelphus schrenkii	KACC42611	KUS-F22135, WU 31234	HQ696660, HQ696652
P. schrenkii	KACC43022	KUS-F22730	HQ696661, HQ696653
P. schrenkii	KACC43136	KUS-F22752, WU 31235	HQ696662, HQ696654
Deutzia parviflora	KACC43189	KUS-F23006, WU 31237	HQ696663, HQ696655
D. parviflora	KACC45741	KUS-F25316, WU 31238	HQ831441, HQ696656

DNA extraction, PCR and sequencing

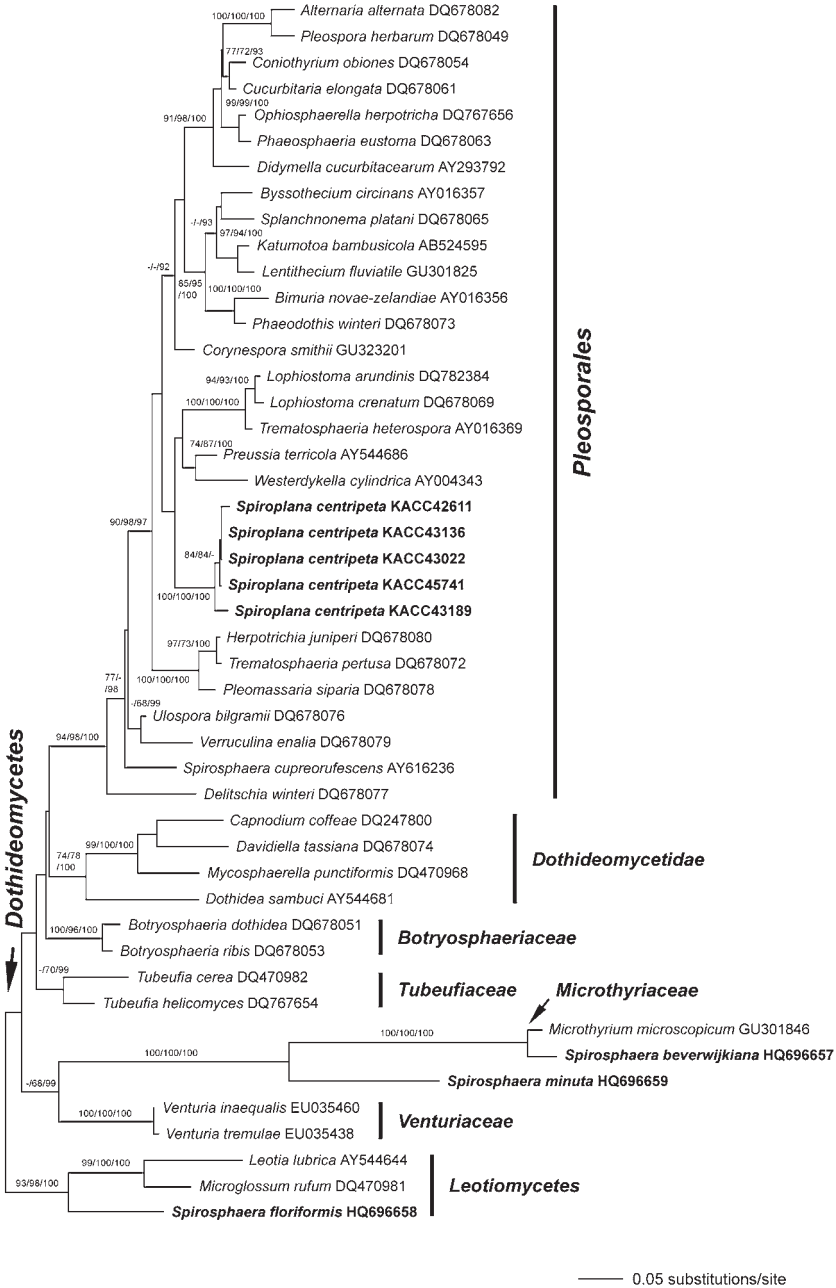
Mycelia harvested from the colonies grown on PDA were used for genomic DNA extraction which was carried out according to Lee & Taylor (1990). The ITS rDNA region of *Spiroplana* was amplified using primers ITS1 and ITS4 (White et al. 1990), and nuLSU rDNA region using primers LROR (Moncalvo et al. 1995) and LR7 (Vilgalys & Hester 1990). The PCR products were purified using a LaboPass PCR purification kit (COSMO Genetech, Seoul, Korea). For *Spirosphaera*, the partial nuSSU - complete ITS - partial nuLSU rDNA region was amplified with primers V9G (De Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990); PCR products were purified using an enzymatic PCR cleanup as described in Voglmayr & Jaklitsch (2008). DNA was cycle-sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Warrington) and the PCR primers; in addition, for the SSU-ITS-LSU fragment the primers ITS4 (White et al. 1990) and LR3 (Vilgalys & Hester 1990) were used. Sequencing was performed on a 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA). The sequence data were edited using the DNASTAR computer package version 5.05 (Lasergene, Madison, WI). The newly obtained ITS and nuLSU rDNA sequences of the five isolates of *Spiroplana* and of the three *Spirosphaera* species were deposited in GenBank.

Data analysis

As a nucleotide BLAST search did not reveal highly similar sequences for the ITS rDNA region of *Spiroplana*, the ITS was not used for phylogenetic analyses. For the phylogenetic analyses of the nuLSU rDNA, representative sequences from *Leotiomycetes* and *Dothideomycetes* were selected from GenBank according to the results of nucleotide BLAST searches of *Spiroplana* and *Spirosphaera* sequences. After a first rough phylogenetic analysis, additional LSU sequences were selected according to the phylogenies of Schoch et al. (2009) and Zhang et al. (2009) to obtain a representative sampling. The outgroup included *Leotia lubrica* (Scop.) Pers., *Microglossum rufum* (Schwein.) Underw., and *Spirosphaera floriformis* (*Leotiomycetes*). GenBank accession numbers of the selected sequences are given in the tree, following the taxon names (Fig. 1). In addition, new and GenBank LSU sequences of verified *Spirosphaera* species were included.

Sequence alignments were generated with Muscle version 3.6 (Edgar 2004) and visually checked and refined with BioEdit (Hall 1999), version 7.0.9.0; excessive leading and trailing gaps were removed. The final LSU matrix contained 1561 characters.

Maximum parsimony (MP) analyses were performed with PAUP* version 4.0 b10 (Swofford 2002), using 1000 replicates of heuristic search with random addition of sequences and subsequent TBR branch swapping (MULTREES option in effect,


Alternaria alternata DQ678082
Pleospora herbarum DQ678049
Coniothyrium obiones DQ678054
Cucurbitaria elongata DQ678061
Ophiosphaerella herpotricha DQ767656
Phaeosphaeria eustoma DQ678063
Didymella cucurbitacearum AY293792
Byssothecium circinans AY016357
Splanchnonema platani DQ678065
Katumotoa bambusicola AB524595
Lentithecium fluviatile GU301825
Bimuria novae-zelandiae AY016356
Phaeodothis winteri DQ678073
Corynespora smithii GU323201
Lophiostoma arundinis DQ782384
Lophiostoma crenatum DQ678069
Trematosphaeria heterospora AY016369
Preussia terricola AY544686
Westerdykella cylindrica AY004343
Spiroplana centripeta KACC42611
Spiroplana centripeta KACC43136
Spiroplana centripeta KACC43022
Spiroplana centripeta KACC45741
Spiroplana centripeta KACC43189
Herpotrichia juniperi DQ678080
Trematosphaeria pertusa DQ678072
Pleomassaria siparia DQ678078
Ulospora bilgramii DQ678076
Verruculina enalia DQ678079
Spirosphaera cupreorufescens AY616236
Delitschia winteri DQ678077
Capnodium coffeae DQ247800
Davidiella tassiana DQ678074
Mycosphaerella punctiformis DQ470968
Dothidea sambuci AY544681
Botryosphaeria dothidea DQ678051
Botryosphaeria ribis DQ678053
Tubeufia cerea DQ470982
Tubeufia helicomyces DQ767654
Microthyrium microscopicum GU301846
Spirosphaera beverwijkiana HQ696657
Spirosphaera minuta HQ696659
Venturia inaequalis EU035460
Venturia tremulae EU035438
Leotia lubrica AY544644
Microglossum rufum DQ470981
Spirosphaera floriformis HQ696658
Pleosporales
Dothideomycetes
Dothideomycetidae
Botryosphaeriaceae
Tubeufiaceae
Microthyriaceae
Venturiaceae
Leotiomycetes
0.05 substitutions/site

COLLAPSE=MAXBRLEN, steepest descent option not in effect). All molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data. Bootstrap analysis with 1000 replicates was performed in the same way, but using 10 rounds of random sequence addition and subsequent branch swapping during each bootstrap replicate.

For maximum likelihood (ML) analyses, 500 rounds of random addition of sequences as well as 500 bootstrap replicates were computed with RAxML version 7.0.4 (Stamatakis 2006) using the GTRMIXI and GTRCAT algorithms, respectively. GTRCAT efficiently approximates the well-known general time reversible model of site substitution combined with a gamma distribution (GTR+G) to accommodate among-site substitution rate heterogeneity. GTRMIXI uses GTRCAT during heuristic search, but the full GTR+I+G model for the final likelihood computation. Best rearrangement settings were estimated by RAxML during tree search.

For Bayesian analyses, the GTR+I+G model was implemented. Bayesian analyses were performed with the computer program MrBayes (version 3.1.2; Huelsenbeck & Ronquist 2001). Three parallel runs of four incrementally heated, simultaneous Markov chains were performed over 5 million generations from which every 200th tree was sampled in each run. The first 1000 trees sampled were discarded, and a 90% majority rule consensus of the remaining trees was computed to obtain estimates for the probabilities that groups are monophyletic given the sequence data (posterior probabilities). To test convergence of runs, the results were analysed using AWTY (Nylander et al. 2008); no indication of lack of convergence was detected.

Results

The final alignments and the trees obtained were deposited in TreeBASE (http://www.treebase.org) and are available at http://purl.org/phylo/treebase/phylows/study/TB2:S11131.

Of the 1561 characters in the nuLSU alignment, 338 were parsimony-informative. Parsimony analysis revealed 133 MP trees consisting of 1580 steps (not shown). Topology of the 133 MP trees differed in the deeper nodes of *Dothideomycetes*, which collapsed to a polytomy up to the *Pleosporales* clade in the strict consensus tree, and within the *Pleosporales*, where several of the deeper nodes of the backbone lacking significant support collapsed to a polytomy as well. Fig. 1 shows the best ML tree (lnL = –9699.653), which is fully compatible with the MP strict consensus tree. Tree topologies of the Bayesian analyses were fully compatible with the ML tree. The three Bayesian runs revealed almost identical posterior probabilities (PP).

Fig. 1. Phylogram of the best ML tree revealed by RAxML from an analysis of the nuLSU rDNA matrix of selected *Dothideomycetes* and *Leotiomycetes*, showing the phylogenetic position of *Spiroplana centripeta* within the *Pleosporales*. GenBank sequence accession numbers or KACC strain numbers (for *Spiroplana centripeta*) follow the taxon names. MP and ML bootstrap support above 65% and Bayesian posterior probabilities above 90% are given at first, second and third position, respectively, above or below the branches. Labels in bold denote sequences obtained during the present study.

In all analyses, *Spiroplana centripeta* was placed in the *Pleosporales* with high support (Fig. 1); however, its closest relatives within the order could not be revealed. All species of the morphologically similar anamorph genus *Spirosphaera* included in the analyses are unrelated to *Spiroplana*; in addition, they are mostly unrelated to each other as well (Fig. 1). Whereas *Spirosphaera floriformis*, the generic type, is placed in the *Leotiomycetes*, the other three species fall into the *Dothideomycetes*. *Spirosphaera beverwijkiana* is closely related to *Microthyrium microscopicum* Desm., whereas *S. minuta* is sister species to the *S. beverwijkiana*/*Microthyrium* clade, but separated by wide genetic distance (Fig. 1). *Spirosphaera cupreorufescens* Voglmayr is embedded within the *Pleosporales*, representing the species of *Spirosphaera* most closely related to *Spiroplana*.

Taxonomy

Spiroplana Voglmayr, M.J. Park & H.D. Shin **gen. nov.**

MycoBank MB 519376

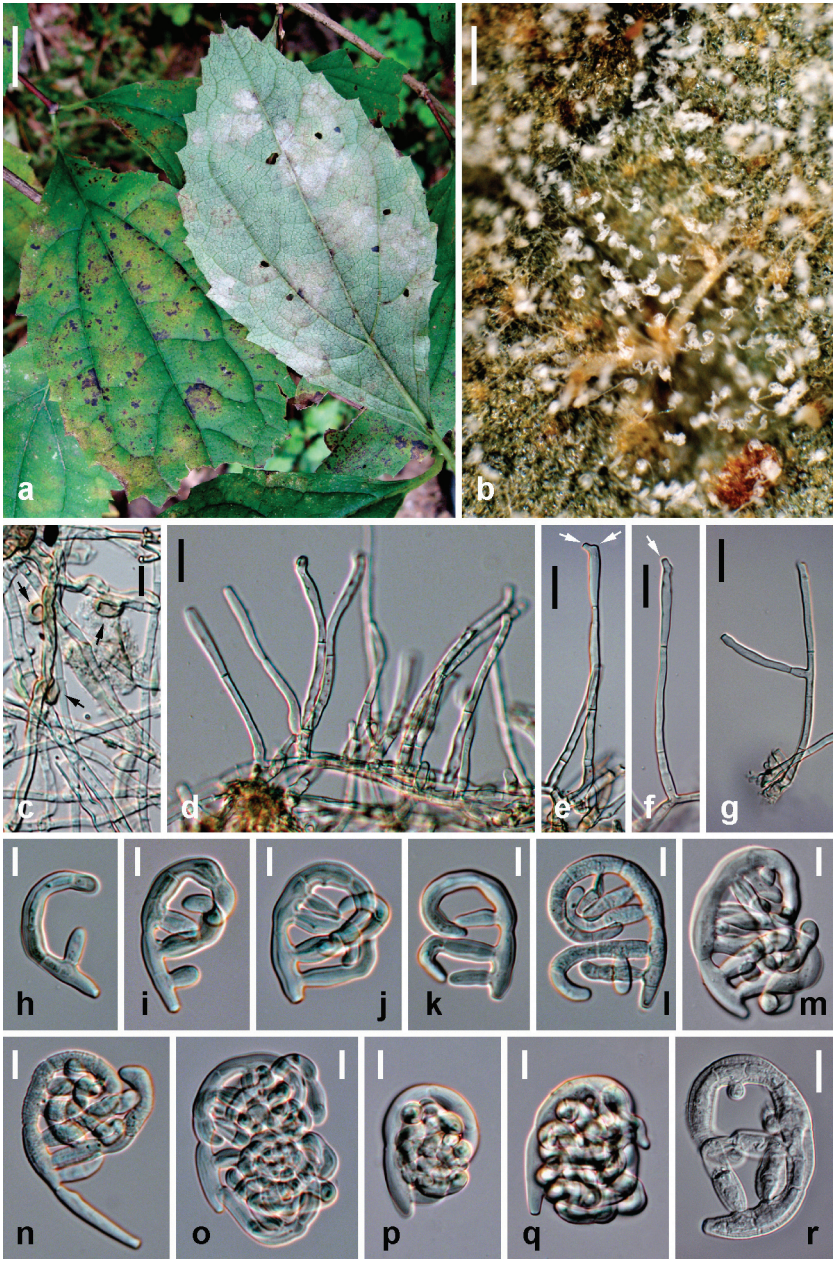
Mycelium parasiticum in foliis vivis, partim epiphyllum, partim endoparasiticum. Hyphae septatae, ramificantes. Conidiophora erecta, mononematosa, septata. Cellulae conidiogenae integratae, holoblasticae, terminales. Conidia irregulariter globosa vel elongata, lateraliter compressa, formantur filamentis spiraliter intertextis ramificantibusque; filamentum primarium centripete gerens filamenta filialia circinata.

Type: *Spiroplana centripeta* Voglmayr, M.J. Park & H.D. Shin

Etymology: Referring to the laterally flattened conidia made up of conidial filaments branching and coiling in one plane and the morphological similarity to *Spirosphaera*.

Mycelium parasitic on leaves, partly superficial, partly endoparasitic. Hyphae septate, branching. Conidiophores erect, mononematous, septate. Conidiogenous cells terminally integrated, holoblastic. Conidia irregularly globose to elongated, laterally flattened, formed by branched, tightly spirally interwoven, septate conidial filaments; the primary conidial filament giving rise to centripetally growing, coiled daughter filaments at the inner side of the coil.

Fig. 2. Disease symptoms and LM of *Spiroplana centripeta*. a. Infected leaves of *Philadelphus schrenkii* from above and below, showing the yellowish green to brownish lesions above and the whitish surface mycelium with abundant sporulation below; black dots are holes caused by insects. b. Detail of infected leaf underneath, showing whitish spores on conidiophores. c. Septate hyaline hyphae in surface mycelium with brownish appressoria (white arrows). d–g. Erect conidiophores arising from superficial mycelium; white arrows denote detachment scars of conidia; d–f unbranched, g branched conidiophores. h–r conidia showing the inward branching of the main filament in one plane and the tight coiling of the densely interwoven daughter filaments; h–l. young, n–r. mature conidia; l, n, r. with verrucose conidial filament; n. with uppermost cell of conidiophore still attached. Sources: a. KUS-F22920, b–r. KUS-F23616. Scale bars: a = 1 cm, b = 200 µm, c, h–r = 10 µm, d–g = 20 µm.


a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
r

Spiroplana centripeta Voglmayr, M.J. Park & H.D. Shin **sp. nov.** Figs. 2–3

MycoBank MB 519377

Mycelium album, partim epiphyllum epidermidem inferiorem foliorum viventium obtegens, partim endoparasiticum. Hyphae hyalinae vel subhyalinae, septatae, 2.5–10 µm latae. Conidiophora macronematosa, mononematosa, hyalina vel subhyalina, septata, non vel rarim ramosa, ca 55–240 µm longa, 3–8.5 µm lata. Cellulae conidiogenae terminales integratae, holoblasticae. Conidia hyalina, irregulariter subglobosa, diametro ca 30–105 µm, lateraliter compressa, formantur filamentis spiraliter intertextis centripete ramificantibusque. Filamenta conidialia hyalina, circinata, distincte septata, ad septa constricta, ca 5–10 µm lata, septis ca 3–9 µm latis; cellulis ca 7.5–30 µm longis, curvatis, saepe unum vel dua filamenta lateralia circinata centripetalia gerentia.

Type: Korea, Chuncheon, Bongmyeong-ri, 37°46′49″N, 127°48′55″E, 270 m a.s.l., on *Philadelphus schrenkii*, 11 Sep. 2006, M.J. Park & H.D. Shin (KUS-F22135 **Holotype**, WU 31234 **Isotype**); ex-type culture KACC42611; ex-type sequences HQ696660 (ITS), HQ696652 (LSU).

Etymology: Referring to the primary coil of the conidial filament branching strictly inwards, with daughter filaments growing towards the centre.

Lesions commonly present on leaves, irregularly polyangular, up to 3 cm in diameter, with indistinct margins, confluent, causing yellowish to brownish green discolouration of the host tissues. Mycelium partly superficial, partly endoparasitic; surface mycelium growing cobweb-like on the abaxial (lower) epidermis of the leaves, connected with the endoparasitic mycelium via the stomata. Hyphae hyaline to subhyaline, septate; superficial hyphae frequently branching, not constricted at the septa, smooth, hyphal cells 2.5–6.5 µm wide, occasionally with thick-walled, brownish, ellipsoid appressoria 8–11 µm wide; endoparasitic hyphae intercellular, branched, knobby, slightly constricted at the septa, smooth, hyphal cells 2.5–10 µm wide. Haustoria not observed. Chlamydospores not observed. Colonies on PDA growing slowly, attaining 10–15 mm diam. after 30 d at 25°C, creamy white, later becoming pale creamy brown with aging, raised, covered by dense, velvety aerial mycelium, with irregular margin, reverse pale yellow, without sporulation. Conidiophores erect, formed on surface hyphae at an angle of c. 90°, macronematous, mononematous, hyaline to subhyaline, septate, not constricted at the septa, mostly unbranched, rarely laterally branched, (56–) 79–160(–237) µm long, (2.8–)3.2–5.6(–8.4) µm wide (n = 28), consisting of 3–5 cells. Conidiogenous cells terminally integrated, holoblastic. Conidial secession schizolytic, scar flat to slightly convex, without thickened cell wall, not pigmented. Conidia white in mass, irregularly subglobose, laterally flattened, (36–)47–74(–106) µm long, (29–)34–54(–73) µm wide (n = 42), formed by branched, tightly spirally interwoven filaments consistently growing inwards. Conidial filaments hyaline, coiled, septate, slightly to strongly constricted at the septa, cells (7.4–)14–25.9(–30.2) µm long, (4.7–)6.9–9.1(–10.2) µm wide, (3.2–)4.3–7(–9.2)

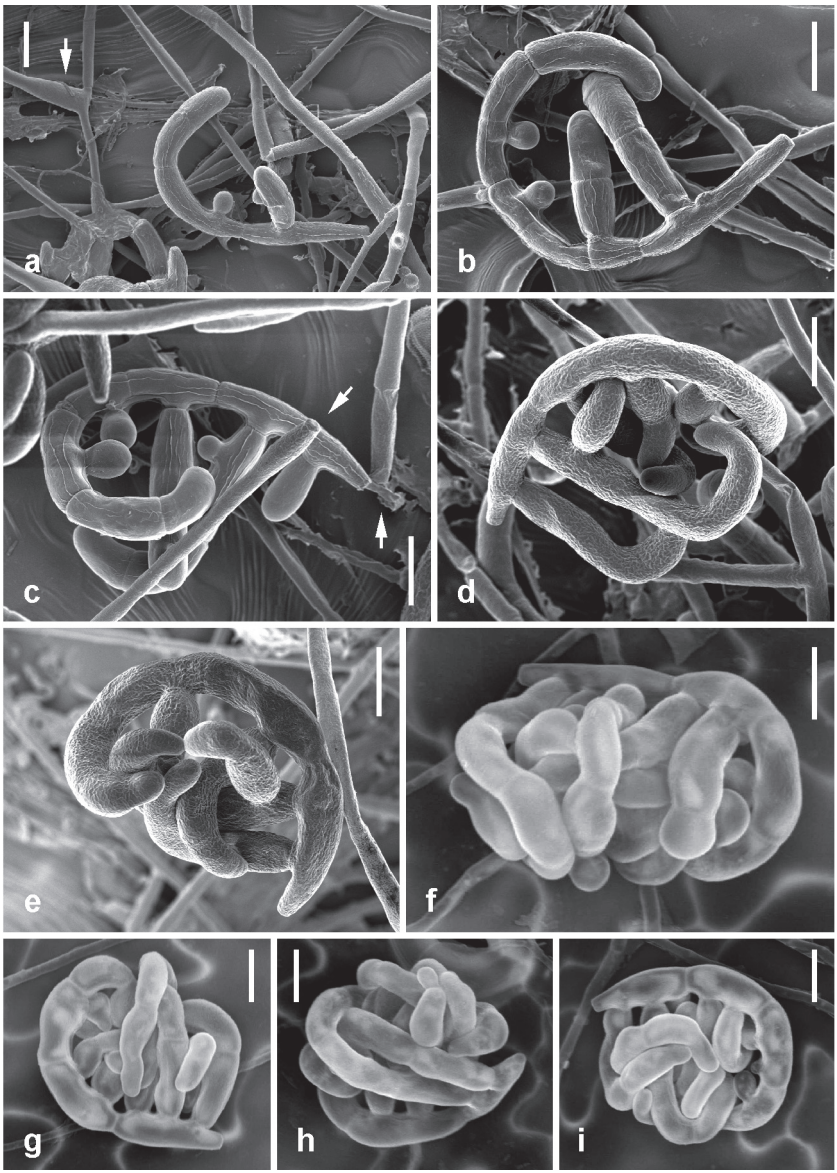


Fig. 3. SEM of conidia of *Spiroplana centripeta* (KUS-F25295). Arrows denote conidiophores. a, b. Detached young conidia and surface mycelium. c–d. Immature conidia. e–i. Mature conidia. Note the spiral conidial filaments branching inwards in one plane and the striate-verrucose surface ornamentation of the conidial filament. Scale bars: 10 μm.

µm at the septa (n = 50); primary filament hook-like, resuming growth after 2/3 to 3/4 of a full coil, giving rise to one or two daughter filaments unilaterally at the inner side of the coil; daughter filaments growing inwards, strongly coiled and tightly interwoven with their neighbour coils, retaining air between their cells.

Distribution: known thus far only from Korea.

Habitat and Host range: parasitic on living leaves of *Philadelphus schrenkii* Rupr. and *Deutzia parviflora* Bunge (*Philadelphaceae*).

Additional specimens examined: On ***Philadelphus schrenkii***: **KOREA.** Chuncheon, Bongmyeong-ri, 37°46′49″N, 127°48′55″E, 270 m a.s.l., 29 Jul. 2004, H.D. Shin (KUS-F20531); 12 Aug. 2006, H.D. Shin (KUS-F21978); 22 Jul. 2007, M.J. Park & H.D. Shin (KUS-F22730); 20 Aug. 2007, M.J. Park & H.D. Shin (KUS-F22778); 4 Sep. 2010, M.J. Park & H.D. Shin (KUS-F25241); 15 Sep. 2010, M.J. Park & H.D. Shin (KUS-F25295); Hoengseong, Hoengseong recreational forest, 37°32′09″N, 127°07′07″E, 230 m a.s.l., 4 Aug. 2004, H.D. Shin (KUS-F20566); 3 Aug. 2007, M.J. Park & H.D. Shin (KUS-F22752, WU 31235); 21 Sep. 2007, M.J. Park & H.D. Shin (KUS-F22920); 27 Jul. 2009, M.J. Park & H.D. Shin (KUS-F24377); Mt. Maebongsan, 37°29′18″N, 127°51′05″E, 220 m a.s.l., 15 Aug. 2008, M.J. Park & H.D. Shin (KUS-F23572); Hongcheon, Bukbang-myeon, 37°48′50″N, 127°50′56″E, 310 m a.s.l., 11 Aug. 2004, H.D. Shin (KUS-F20599); 24 Aug. 2004, H.D. Shin (KUS-F20645); Yeonhwasa temple, 37°48′02″N, 127°51′03″E, 290 m a.s.l., 18 Sep. 2004, H.D. Shin (KUS-F20724); 21 Aug. 2008, M.J. Park & H.D. Shin (KUS-F23599); Gangwon natural environment research park, 37°44′41″N, 127°51′59″E, 230 m a.s.l., 4 Aug. 2006, H.D. Shin (KUS-F21958); Sutasa temple, 37°41′59″N, 127°57′43″E, 195 m a.s.l., 16 Jul. 2009, M.J. Park & H.D. Shin (KUS-F24322); Goesan, Ihwaryeong, 36°45′07″N, 128°01′56″E, 540 m a.s.l., 20 Sep. 2009, M.J. Park & H.D. Shin (KUS-F24642); Yanggu, Dong-myeon, 38°10′05″N, 127°03′23″E, 360 m a.s.l., 24 Sep. 2009, M.J. Park & H.D. Shin (KUS-F24668); Pyeongchang, Jangjeon-ri, 37°30′02″N, 128°33′40″E, 460 m a.s.l., 26 Sep. 2010, M.J. Park & H.D. Shin (KUS-F25315); Jeongseon, Aesan-ri, 37°22′19″N, 128°40′02″E, 340 m a.s.l., 26 Sep. 2010, M.J. Park & H.D. Shin (KUS-F25328); Yangpyeong, Jungmisan recreational forest, 37°35′45″N, 127°28′08″E, 724 m a.s.l., 28 Aug. 2008, M.J. Park & H.D. Shin (KUS-F23616, WU 31236). On ***Deutzia parviflora***: **KOREA**. Hoengseong, Seowon-myeon, 37°31′33″N, 127°52′29″E, 285 m a.s.l., 15 Oct. 2007, M.J. Park & H.D. Shin (KUS-F23006, WU 31237); 15 Aug. 2008, M.J. Park & H.D. Shin (KUS-F23571); Pyeongchang, Jangjeon-ri, 37°30′02″N, 128°33′40″E, 460 m a.s.l., 26 Sep. 2010, M.J. Park & H.D. Shin (KUS-F25316, WU 31238).

Discussion

The conidia of *Spiroplana* most closely resemble those of the anamorph genus *Spirosphaera* in having coiled, branched, tightly interwoven conidial filaments, with usually only a single daughter filament per filament cell (unilateral branching; Hennebert 1998). However, the conidial morphology of *Spiroplana* differs in detail by the consistently centripetal growth of the daughter filaments and the laterally flattened conidia, which is not observed in *Spirosphaera*. Also the genus *Clathrosporium* Nawawi & Kuthub. shows some similarities to *Spiroplana* in having coiled, branched, tightly interwoven conidial filaments; however, the former differs by opposite (bilateral) branching of its conidial filament, and by conidia disarticulating at age (Hennebert 1998).

In addition, ecology is also markedly different: Whereas *Spiroplana* is a pathogen of living leaves of *Philadelphaceae*, members of *Spirosphaera* and *Clathrosporium* are aquatic saprotrophs. Ecologically, *Spirosphaera* and *Clathrosporium* species belong to the aeroaquatic fungi (Voglmayr 2004), which are characterised by growth on submerged litter in stagnant or slow-flowing water bodies and by the production of buoyant propagules (Webster & Descals 1981, Michaelides & Kendrick 1982). Most members of this group produce complex multicellular conidia only above the water level, which enclose air between their cells, are hydrophobic and therefore buoyant. Dispersal of buoyant conidia is on the water surface, where they attach to floating litter. Production of such multicellular dispersal units has evolved multiple times independently in various lineages of ascomycetes. This is evident also in *Spirosphaera*, the members of which are not closely related but highly polyphyletic (Fig. 1), which requires a revised taxonomy of the group. However, this will only be possible after detailed morphological and molecular phylogenetic studies, which are not yet available.

The astounding similarities of the conidia of *Spiroplana* to those of aeroaquatic species and the independent evolution of similar conidia indicate the presence of similar functional constraints, despite their highly different habitats and ecology. These are likely to be found in the dispersal of buoyant conidia on a water film, and in an enhanced attachment of the large conidia on the substrate enabling rapid colonisation by numerous germination hyphae. Conidia of *Spiroplana* are evidently not adapted to wind-dispersal as they are comparatively large, compact and do not have appendages. *Spiroplana* has been found in areas characterised by humid climate and frequent rainfalls in summer in the vicinity of streams, where dispersal on a water film, dew or via splash drops appears likely, although this has not yet been observed in nature. A similar example of a leaf parasite with multicellular complex conidia reminiscent of aeroaquatic fungi concerns the anamorph genus *Cristulariella depraedans* (Cooke) Höhn., a widespread leaf spot pathogen of *Acer* spp. (Redhead 1975, Voglmayr & Delgado-Rodríguez 2003, Narumi-Saito et al. 2006). Comparable cases of spore adaptation to water dispersal have also been reported for some common terrestrial foliicolous fungi producing tetraradiate conidia (e.g. *Tripospermum* Speg., see Tubaki et al. 1985), which are morphologically similar to those of Ingoldian aquatic fungi inhabiting leaf litter in rapidly flowing streams (Gönczöl & Révay 2006). In aquatic fungi, tetraradiate spores have been shown to be an adaptation to improved and accelerated attachment to the substrate, a critical issue for successful substrate colonisation in rapidly flowing water bodies (Read et al. 1992). The same dispersal constraints may also apply to foliicolous fungi in humid climates, resulting in the evolution of similar morphological adaptations in spore shape. A recently detected example of a leaf pathogen with

tetraradiate conidia similar to Ingoldian fungi is *Miricatena prunicola* Punith. & Spooner, the agent of a leaf-spot disease of *Prunus serotina* (Punithalingam & Spooner 2011). However, also in this case the dispersal mechanisms remain to be investigated in detail.

Spiroplana centripeta is only known from a comparatively small area within Korea, with *Philadelphus schrenkii* and *Deutzia parviflora* as only confirmed hosts. Considering the distribution area of its hosts, which extends to central China and south-eastern Russia, *S. centripeta* may have a wider distribution in East Asia but may have escaped notice due to the rather inconspicuous disease symptoms. It appears possible that additional hosts from *Philadelphaceae* are susceptible to infection. As several species of *Philadelphus* and *Deutzia* are commonly planted as ornamental shrubs in temperate climates world-wide, the pathogen is of potential horticultural concern. However, considering the limited disease symptoms observed in the present study, it is unlikely to become a major threat.

Acknowledgements

We gratefully acknowledge Walter Gams and Roland Kirschner for pre-submission reviews of the manuscript and Brian Spooner for providing information on *Miricatena*. HV is grateful to SYNTHESYS grant NL-TAF-1915 for funding a research visit to CBS. Financial support was provided by the KIÖS of the Austrian Academy of Sciences (grant P2008-02). This work was also supported by a grant from Regional Subgenebank Support Program of Rural Development Administration, Republic of Korea.

Literature cited

Clémençon H. 1972. Zwei verbesserte Präparierlösungen für die mikroskopische Untersuchung von Pilzen. Zeitschrift für Pilzkunde 38: 49–53.

De Hoog GS, Gerrits van den Ende AHG. 1998. Molecular diagnostics of clinical strains of filamentous *Basidiomycetes*. Mycoses 41: 183–189. doi:10.1111/j.1439-0507.1998.tb00321.x

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797. doi:10.1093/nar/gkh340

Gönczöl J, Révay A. 2006. Species diversity of rainborne hyphomycete conidia from living trees. Fungal Diversity 22: 37–54.

Halbritter H. 1998. Preparing living pollen material for scanning electron microscopy using 2,2-Dimethoxypropane (DMP) and critical-point drying. Biotechnic & Histochemistry 73: 137–143. doi:10.3109/10520299809140519

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hennebert GL. 1998. New species of the aeroaquatic hyphomycete genus *Clathrosporium* and their relationship with *Strumella* Sacc. Canadian Journal of Botany 76: 1596–1607. doi:10.1139/cjb-76-9-1596

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755. doi:10.1093/bioinformatics/17.8.754

Lee SB, Taylor JW. 1990. Isolation of DNA from fungal mycelia and single spores. 282–287, in: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds.): PCR Protocols: a Guide to Methods and Applications. Academic Press, San Diego.

Michaelides J, Kendrick B. 1982. The bubble-trap propagules of *Beverwykella*, *Helicoon* and other aero-aquatic fungi. Mycotaxon 14: 247–260.

Moncalvo JM, Wang HH, Hseu RS. 1995. Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacers and 25S ribosomal DNA sequences. Mycologia 87: 223–238. doi:10.2307/3760908

Narumi-Saito T, Hosoya T, SanoT, Harada Y. 2006. *Nervostroma*, gen. nov. in the *Sclerotiniaceae*, the teleomorph of *Cristulariella*, and *Hinomyces* anam. gen. nov. to accommodate the anamorph of *Grovesinia*: reassessment of the genus *Cristulariella*. Mycoscience 47: 351–359. doi:10.1007/s10267-006-0313-9

Nylander JA, Wilgenbusch JC, Warren DL, Swofford DL. 2008. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24: 581–583. doi:10.1093/bioinformatics/btm388

Punithalingam E, Spooner BM. 2011. *Miricatena prunicola* (hyphomycetes), a new genus and species causing leaf spots of *Prunus serotina* in UK. Kew Bulletin (in press)

Read SJ, Moss ST, Jones EBG. 1992. Attachment and germination of conidia. 135–151, in: Bärlocher F (ed.): The Ecology of Aquatic Hyphomycetes. Springer, Berlin.

Redhead SA. 1975. The genus *Cristulariella*. Canadian Journal of Botany 53: 700–707.

Schoch CL, Crous PW, Groenewald JZ, Boehm EWA, Burgess TI, de Gruyter J, De Hoog GS, Dixon LJ, Grube M, Gueidan C, Harada Y, Hatakeyama S, Hirayama K, Hosoya T, Huhndorf SM, Hyde KD, Jones EBG, Kohlmeyer J, Kruys Å, Li YM, Lücking R, Lumbsch HT, Marvanová L, Mbatchou JS, McVay AH, Miller AN, Mugambi GK, Muggia L, Nelsen MP, Nelson P, Owensby CA, Phillips AJL, Phongpaichit S, Pointing SB, Pujade-Renaud V, Raja HA, Rivas Plata E, Robbertse B, Ruibal C, Sakayaroj J, Sano T, Selbmann L, Shearer CA, Shirouzu T, Slippers B, Suetrong S, Tanaka K, Volkmann-Kohlmeyer B, Wingfield MJ, Wood AR, Woudenberg JHC, Yonezawa H, Zhang Y, Spatafora JW. 2009. A class-wide phylogenetic assessment of *Dothideomycetes*. Studies in Mycology 64: 1–15. doi:10.3114/sim.2009.64.01

Stamatakis E. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. doi:10.1093/bioinformatics/btl446

Swofford DL. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods), Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Tubaki K, Tokumasu S, Ando K. 1985. Morning dew and *Tripospermum* (*Hyphomycetes*). Botanical Journal of the Linnean Society 91: 45–50. doi:10.1111/j.1095-8339.1985.tb01133.x

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238–4246.

Voglmayr H. 2004. *Spirosphaera cupreorufescens* sp. nov., a rare aeroaquatic fungus. Studies in Mycology 50: 221–228.

Voglmayr H, Delgado-Rodríguez G. 2003. New species, notes and key to the aeroaquatic genera *Beverwykella* and *Ramicephala* gen. nov. Mycological Research 107: 236–244. doi:10.1017/S0953756203007172

Voglmayr H, Jaklitsch WM. 2008. *Prosthecium* species with *Stegonsporium* anamorphs on *Acer*. Mycological Research 112: 885–905. doi:10.1016/j.mycres.2008.01.020

Webster J, Descals E. 1981. Morphology, distribution, and ecology of conidial fungi in freshwater habitats. 295–355, in: Cole GT, Kendrick B (eds.): Biology of Conidial Fungi, vol. 1. Academic Press, New York.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: Innis MA, Gelfand DH, Sninsky JJ, White TJ, (eds): PCR Protocols: a Guide to Methods and Applications. Academic Press, San Diego, California, USA.

Zhang Y, Schoch CL, Fournier J, Crous PW, Gruyter J de, Woudenberg JHC, Hirayama K, Tanaka K, Pointing SB, Spatafora JW, Hyde KD. 2009. Multi-locus phylogeny of the *Pleosporales*: a taxonomic, ecological and evolutionary re-evaluation. Studies in Mycology 64: 85–102. doi:10.3114/sim.2009.64.04